

Figure 1: 1/2

Sequence of the *Archaeoglobus fulgidus* exonuclease III gene:

SEQ ID NO.: 20/21

ATGCTCAAAATCGCCACCTTCAACGTAAACTCCATCAGGAGCAGACTGCACATCGTGATT  
1 -----+-----+-----+-----+-----+ 60  
TACGAGTTTTAGCGGTGGAAGTTGCATTTGAGGTAGTCCTCGTCTGACGTGTAGCACTAA  
a M L K I A T F N V N S I R S R L H I V I -  
CCGTGGCTGAAGGAGAACAAGCCTGACATTCTATGCATGCAGGAGACGAAGGTTGAGAAC  
61 -----+-----+-----+-----+-----+ 120  
GGCACCGACTTCCTCTTGTTGCGACTGTAAGATACGTACGTCCTCTGCTTCCAACCTCTTG  
a P W L K E N K P D I L C M Q E T K V E N -  
AGGAAGTTTCCTGAGGCCGATTTTCACCGCATCGGCTACCACGTCGTCTTCAGCGGGAGC  
121 -----+-----+-----+-----+-----+ 180  
TCCTTCAAAGGACTCCGGCTAAAAGTGGCGTAGCCGATGGTGCAGCAGAAGTCGCCCTCG  
a R K F P E A D F H R I G Y H V V F S G S -  
AAGGGAAGGAATGGAGTGGCCATAGCTTCCCTCGAAGAGCCTGAGGATGTCAGCTTCGGT  
181 -----+-----+-----+-----+-----+ 240  
TTCCCTTCCTTACCTCACCGGTATCGAAGGGAGCTTCTCGGACTCCTACAGTCGAAGCCA  
a K G R N G V A I A S L E E P E D V S F G -  
CTCGATTTCAGAGCCGAAGGACGAGGACAGGCTGATAAGGGCAAAGATAGCTGGCATAGAC  
241 -----+-----+-----+-----+-----+ 300  
GAGCTAAGTCTCGGCTTCCTGCTCCTGTCCGACTATTCCCGTTTCTATCGACCGTATCTG  
a L D S E P K D E D R L I R A K I A G I D -  
GTGATTAACACCTACGTTTCCTCAGGGATTCAAAATTGACAGCGAGAAGTACCAGTACAAG  
301 -----+-----+-----+-----+-----+ 360  
CACTAATTGTGGATGCAAGGAGTCCCTAAGTTTTAACTGTCGCTCTTCATGGTCATGTTT  
a V I N T Y V P Q G F K I D S E K Y Q Y K -  
CTCCAGTGGCTTGAGAGGCTTTACCATTACCTTCAAAAAACCGTTGACTTCAGAAGCTTT  
361 -----+-----+-----+-----+-----+ 420  
GAGGTCACCGAACTCTCCGAAATGGTAATGGAAGTTTTTTGGCAACTGAAGTCTTCGAAA  
a L Q W L E R L Y H Y L Q K T V D F R S F -  
GCTGTTTGGTGTGGAGACATGAACGTTGCTCCTGAGCCAATCGACGTTCACTCCCCAGAC  
421 -----+-----+-----+-----+-----+ 480  
CGACAAACCACACCTCTGTACTTGCAACGAGGACTCGGTTAGCTGCAAGTGAGGGGCTG  
a A V W C G D M N V A P E P I D V H S P D -  
AAGCTGAAGAACCACGTCTGCTTCCACGAGGATGCGAGAAGGGCATACAAAAAATACTC  
481 -----+-----+-----+-----+-----+ 540  
TTCGACTTCTTGGTGCAGACGAAGGTGCTCCTACGCTCTTCCCGTATGTTTTTTATGAG  
a K L K N H V C F H E D A R R A Y K K I L -

a

541

601.

661

721

L

[illegible]

Figure 2:

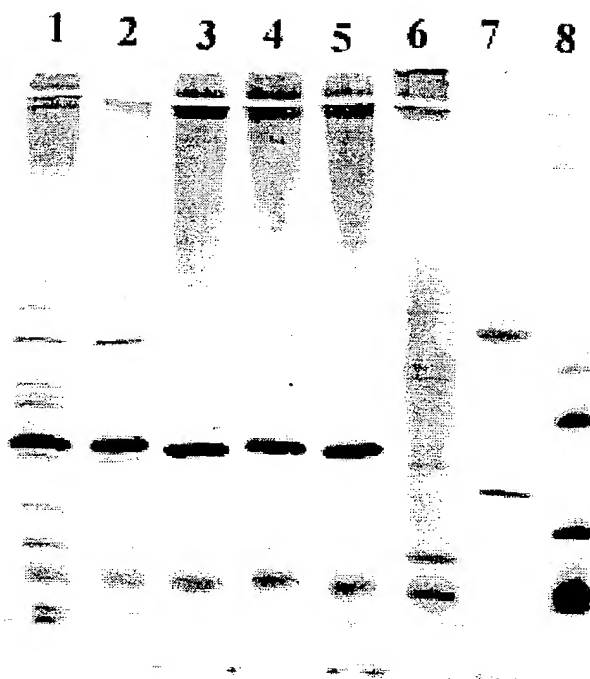
Temperature stability of *Afu* exonuclease III

Figure 3:

Test for exonuclease III activity

1 2 3 4 5 6 7 8 9 10 11 12 13 14

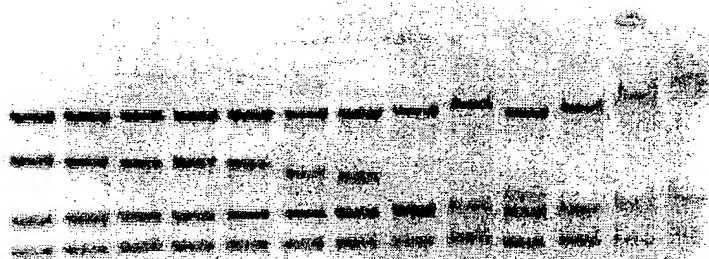
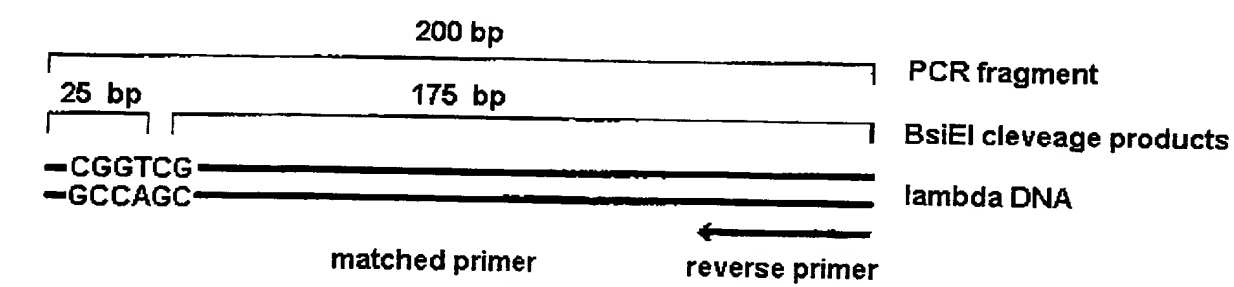


Figure 4:

# Principle of the 3'-primer correction assay



- 1 → G
- 2 → A
- 3 → T
- 4 → C
- 5 → AT

mismatched Primer

↓  
Amplification with Taq DNA polymerase  
in presence of Afu-ExoIII

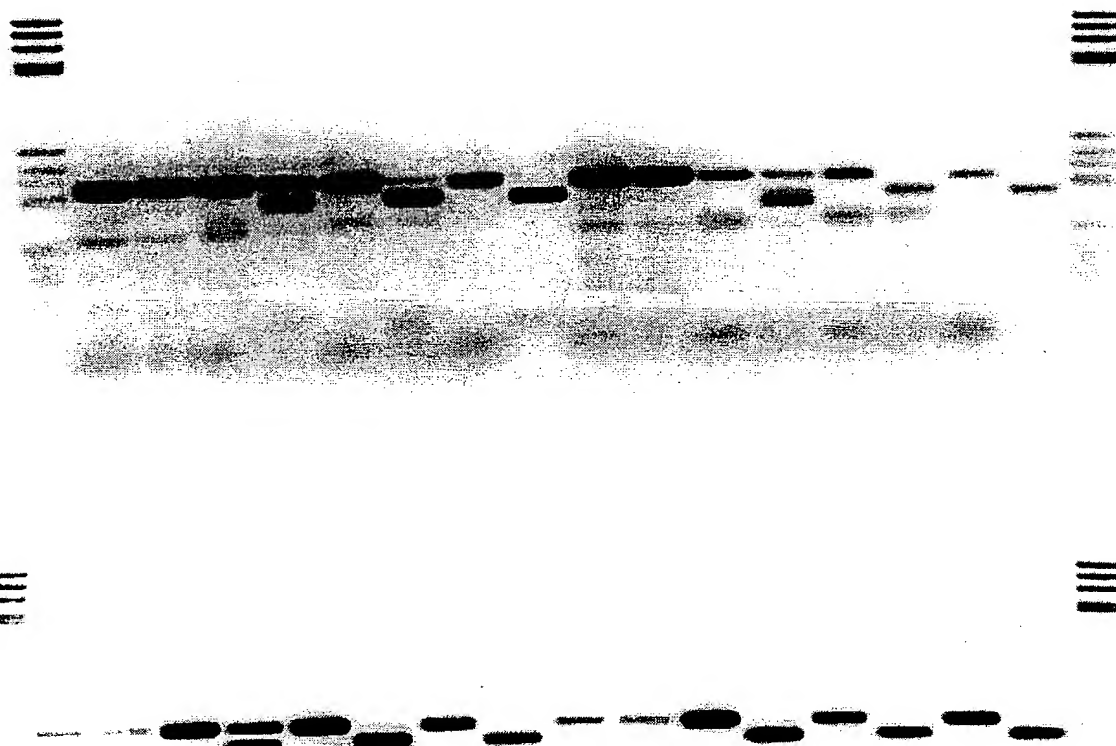
↓  
Digestion with restriction enzyme BsiEI  
Cleavage site CG Pu Py / CG

↓  
separation on agarose gel (1%)

Figure 5:

Mismatched primer correction in PCR

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18



19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Figure 6A

Error rates of different DNA polymerases in PCR

Polymerase	Template conc. (ng)	yield (ng)	DNA duplica- tions d	blue colonies lacI <sup>-</sup>	white colonies lac <sup>+</sup>	total number of colonies	% lac <sup>+</sup>	error rate (f <sub>349</sub> )
Taq Ch.	10	11650	10.2	130	2261	2391	5.4	1.57 x 10 <sup>-3</sup>
HiFi Ch.	10	11550	10.2	40	5458	5498	0.72	2.06 x 10 <sup>-3</sup>
Pwo	10	9675	9.9	17	5891	5908	0.29	8.32 x 10 <sup>-7</sup>
Taq/Exo 1	10	11550	10.2	94	4291	4385	2.14	6.10 x 10 <sup>-5</sup>
Taq/Exo 2	10	11125	10.1	146	7644	7790	1.87	5.36 x 10 <sup>-5</sup>
Taq/Exo 3	10	8500	9.7	133	8188	8321	1.6	4.74 x 10 <sup>-5</sup>
Taq/Exo 4	10	1292	7	79	7236	7315	1.08	4.44 x 10 <sup>-5</sup>
Taq/Exo 5	10	236	4.6	25	2674	2724	0.92	1.16 x 10 <sup>-5</sup>

\* Due to the unfavorable ratio of Taq:Exo the product yield was low. This results in an apparently low amplification efficiency d, which is an important parameter in the formula used for the calculation of the error rate.

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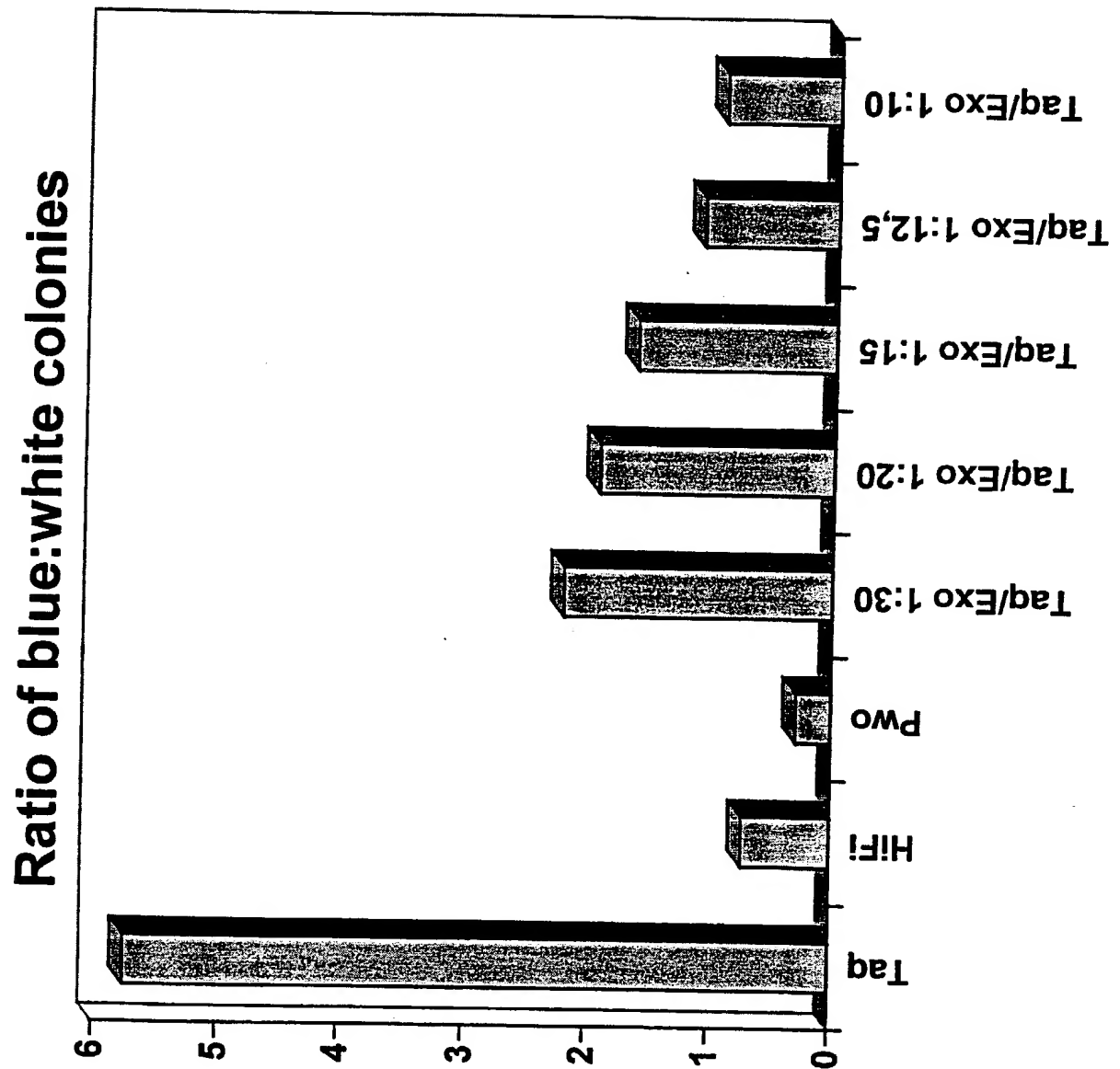
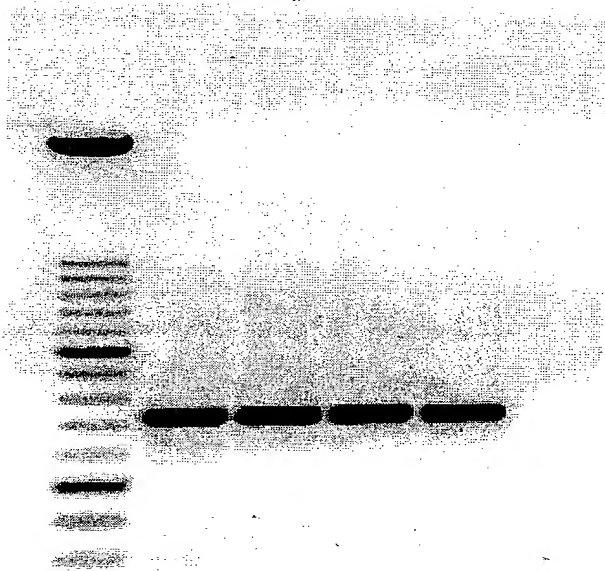


Figure 6B:



Figure 7:

1 2 3 4 5 6



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Figure 8:

UNG treatment of dUMP containing PCR products

1 2 3 4 5 6 7 8 9

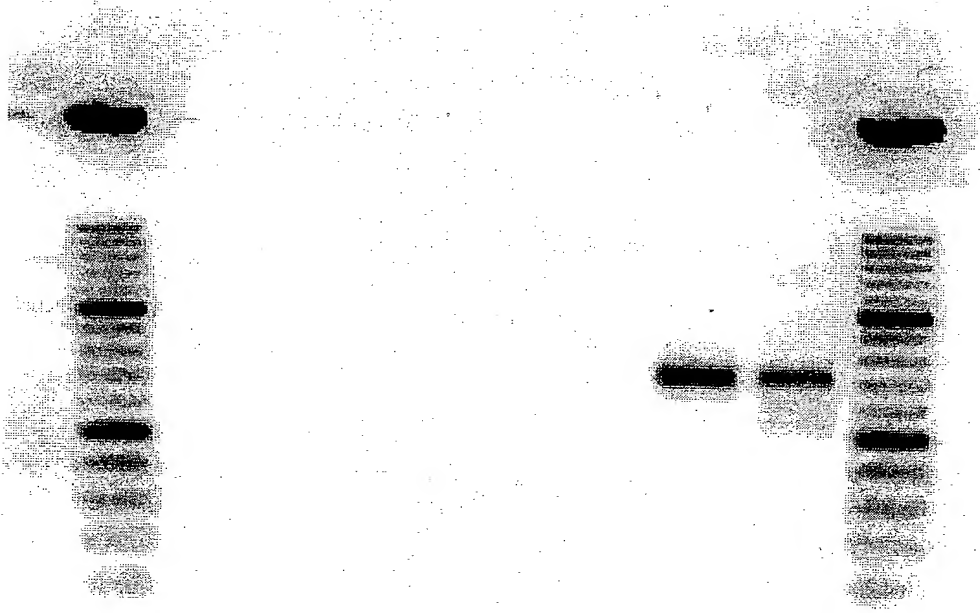


Figure 9:

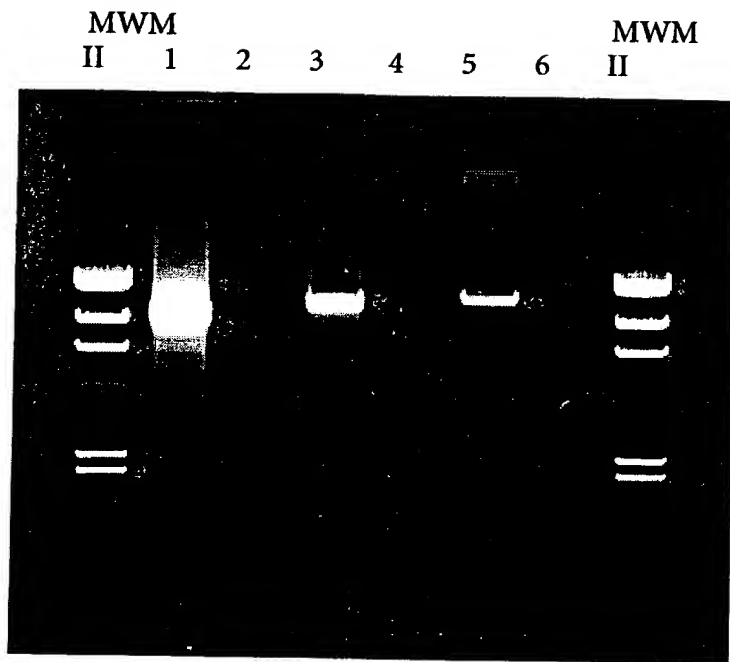


Figure 10:

1	2	3	4	5	6	7	8	9	10
MWM	1	2	3	4	5	6	7	8	MWM

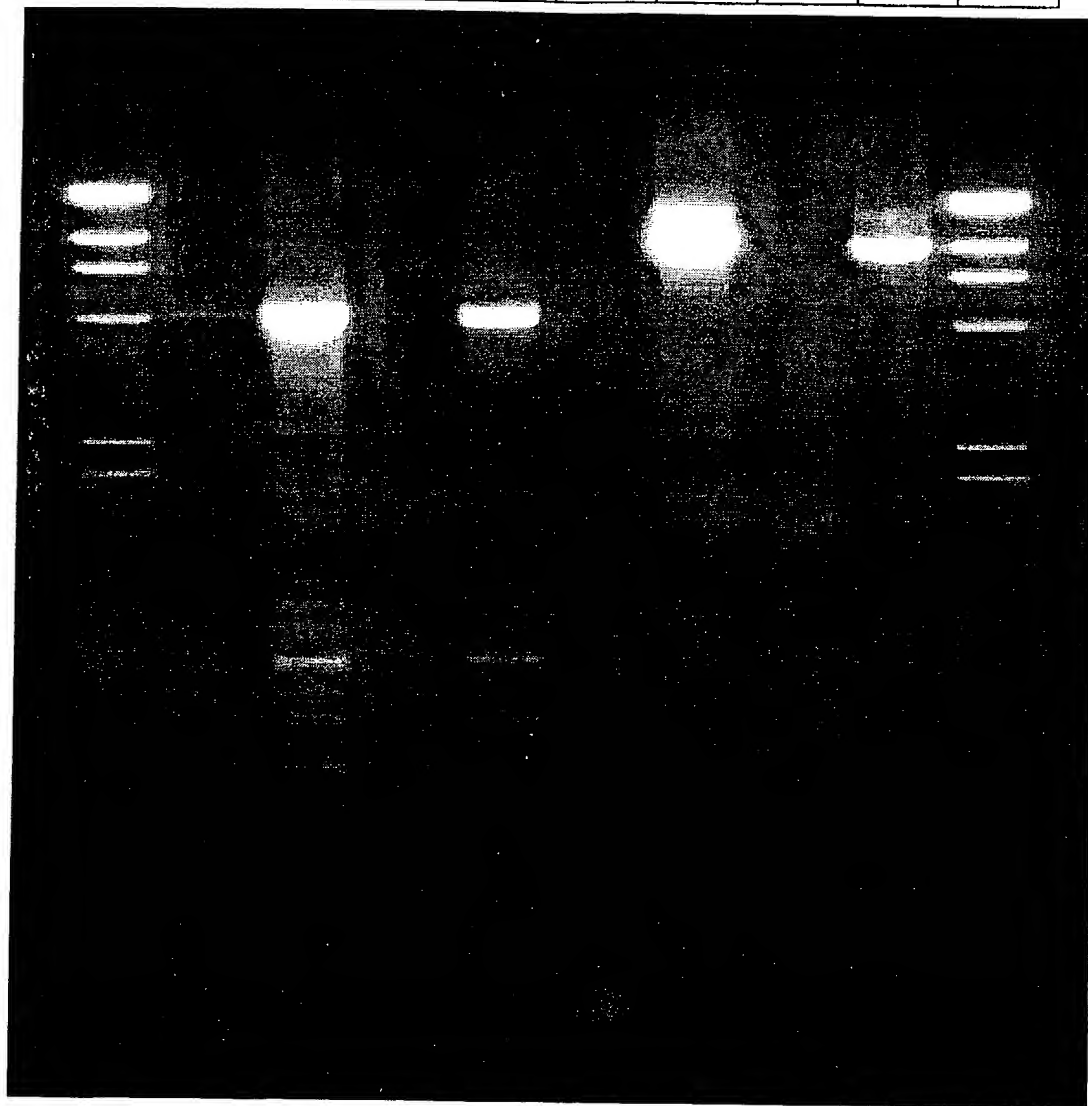
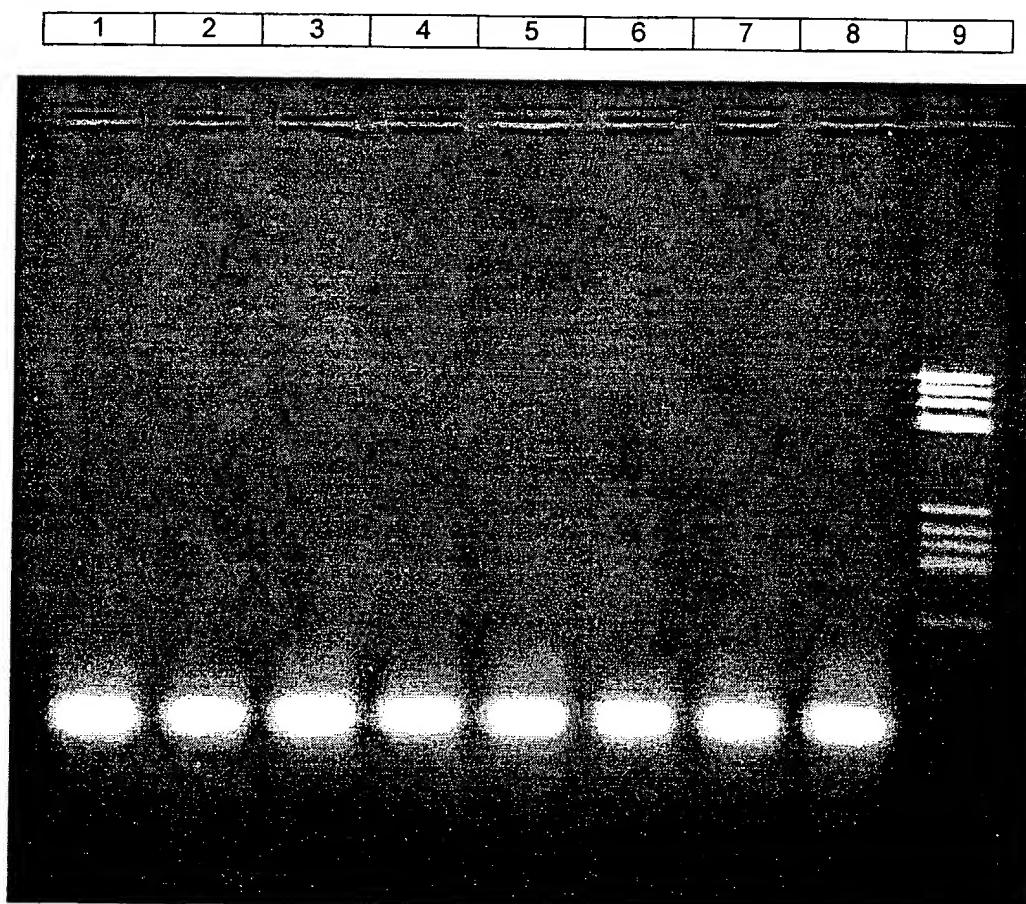
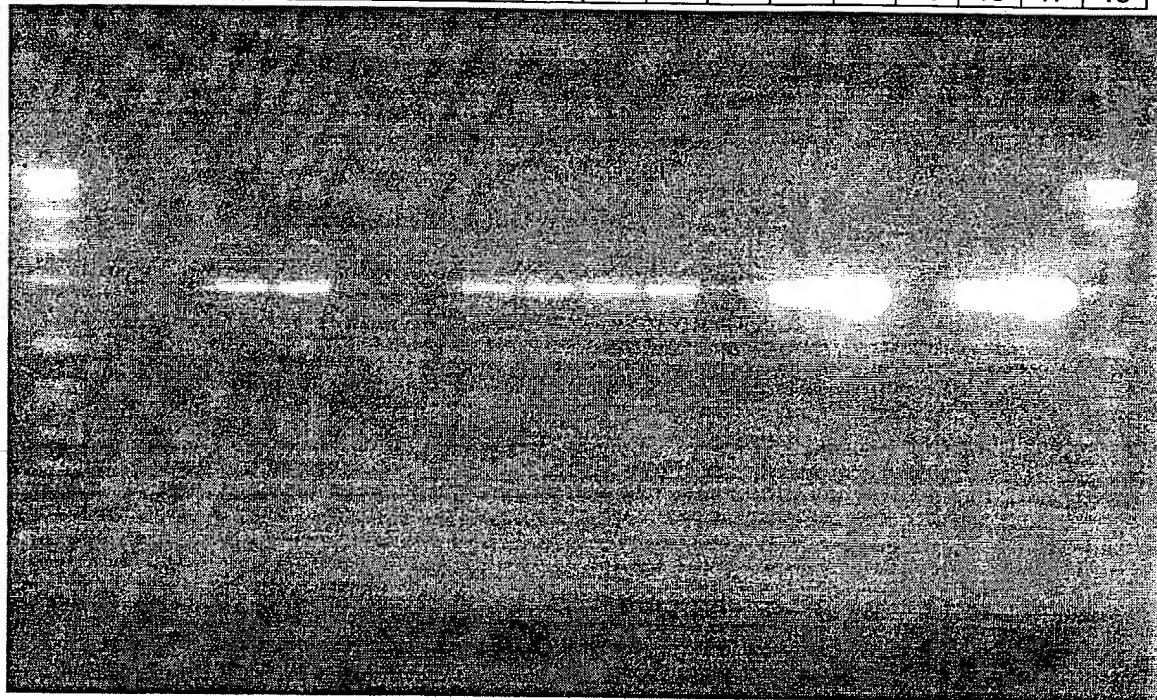


Figure 11



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
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[illegible]